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(54) Title: ZCYTOR7 CYTOKINE RECEPTOR			
(57) Abstract			
<p>Novel cytokine receptor polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed. The polypeptides comprise an extracellular domain of a cell-surface receptor that is expressed in kidneys, pancreas, prostate, adrenal cortex and nervous tissue. The polypeptides may be used within methods for detecting ligands that promote the proliferation and/or differentiation of these organs.</p>			
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## ZCYTOR7 CYTOKINE RECEPTOR

## BACKGROUND OF THE INVENTION

Cytokines are soluble proteins that influence  
10 the growth and differentiation of many cell types. Their  
receptors are composed of one or more integral membrane  
proteins that bind the cytokine with high affinity and  
transduce this binding event to the cell through the  
cytoplasmic portions of the certain receptor subunits.  
15 Cytokine receptors have been grouped into several classes  
on the basis of similarities in their extracellular ligand  
binding domains. For example, the receptor chains  
responsible for binding and/or transducing the effect of  
interferons (IFNs) are members of the type II cytokine  
20 receptor family (CRF2), based upon a characteristic 200  
residue extracellular domain. The demonstrated *in vivo*  
activities of these interferons illustrate the enormous  
clinical potential of, and need for, other cytokines,  
cytokine agonists, and cytokine antagonists.

25

## SUMMARY OF THE INVENTION

The present invention fills this need by  
providing novel cytokine receptors and related  
30 compositions and methods. In particular, the present  
invention provides for an extracellular ligand-binding  
region of a mammalian Zcytor7 receptor, alternatively also  
containing either a transmembrane domain or both an  
intracellular domain and a transmembrane domain.

35

Within one aspect, the present invention  
provides an isolated polynucleotide encoding a ligand-

binding receptor polypeptide. The polypeptide comprises a sequence of amino acids selected from the group consisting of (a) SEQ ID NO:13, residues 30 through 250 of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b). Within one embodiment, the polypeptide comprises the amino acid sequence defined by SEQ ID NO: 13 or amino acid residues 30 through 250 of SEQ ID NO:2. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises a transmembrane domain. The transmembrane domain may comprise residues 251 through 274 of SEQ ID NO:2, or an allelic variant thereof. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises an intracellular domain, such as an intracellular domain comprising residues 275 through 553 of SEQ ID NO:2, or an allelic variant thereof. Within further embodiments, the polynucleotide encodes a polypeptide that comprises residues 1 through 553, 1 through 274, 1 through 250, 30 through 274 or 30 through 553 of SEQ ID NO:2. Within an additional embodiment, the polypeptide further comprises an affinity tag. Within a further embodiment, the polynucleotide is DNA. Also claimed are the isolated polypeptides encoded by these polynucleotides.

25

Within a second aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding a ligand-binding receptor polypeptide, wherein the ligand-binding receptor polypeptide comprises a sequence of amino acids selected from the group consisting of: (i) residues 30 through 250 of SEQ ID NO:2; (ii) allelic variants of (i); and (iii) sequences that are at least 80% identical to (i) or (ii); and (c) a transcription terminator, wherein the promoter, DNA segment, and terminator are operably linked. The ligand-binding

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receptor polypeptide may further comprise a secretory peptide, a transmembrane domain, a transmembrane domain and an intracellular domain, or a secretory peptide, a transmembrane domain and an intracellular domain.

5

Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above, wherein said cell expresses a receptor polypeptide encoded by the DNA segment. Within one embodiment, the cell further expresses a necessary receptor subunit which forms a functional receptor complex. Within another embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for proliferation.

15

Within a fourth aspect of the invention there is provided an isolated polypeptide comprising a sequence selected from the group consisting of (a) residues 30, a valine, through residue 250, a lysine, of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b), wherein said polypeptide is substantially free of transmembrane and intracellular domains ordinarily associated with hematopoietic receptors. Also claimed are polypeptides comprised of a sequence defined by residues 30, a valine, through residue 274, a tyrosine; and a polypeptide comprised of a sequence defined by residues 30, a valine, through residue 553 an asparagine. Also claimed are the polypeptides and polynucleotides defined by the sequences of SEQ ID NOs: 13 - 60.

30

Within a further aspect of the invention there is provided a chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond. The first portion of the chimeric polypeptide consists essentially of a ligand binding

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domain of a receptor polypeptide selected from the group consisting of (a) a receptor polypeptide as shown in SEQ ID NO:2; (b) allelic variants of SEQ ID NO:2; and (c) receptor polypeptides that are at least 80% identical to (a) or (b). The second portion of the chimeric polypeptide consists essentially of an affinity tag. Within one embodiment the affinity tag is an immunoglobulin F<sub>C</sub> polypeptide. The invention also provides expression vectors encoding the chimeric polypeptides and host cells transfected to produce the chimeric polypeptides.

The present invention also provides for an isolated polynucleotide encoding a polypeptide selected from a group defined SEQ ID NO:2 consisting of residues 1 through 250, residues 1 through 274, residues 1 through 553, residues 2 through 250, residues 2 through 274, residues 2 through 553, residues 251 through 274, residues 251 through 553 and residues 275 through 553. Also claimed are the isolated polypeptide expressed by these polynucleotides.

The invention also provides a method for detecting a ligand within a test sample, comprising contacting a test sample with a polypeptide as disclosed above, and detecting binding of the polypeptide to ligand in the sample. Within one embodiment the polypeptide further comprises transmembrane and intracellular domains. The polypeptide can be membrane bound within a cultured cell, wherein the detecting step comprises measuring a biological response in the cultured cell. Within another embodiment, the polypeptide is immobilized on a solid support.

Within an additional aspect of the invention there is provided an antibody that specifically binds to a

polypeptide as disclosed above, as well as an anti-idiotypic antibody which binds to the antigen-binding region of an antibody to Zcytor7.

5 In still another aspect of the present invention, polynucleotide primers and probes are provided which can detect mutations in the Zcytor7 gene. The polynucleotide probe should at least be 20-25 bases in length, preferably at least 50 bases in length and most  
10 preferably about 80 to 100 bases in length. In addition to the detection of mutations, these probes can be used to discover the Zcytor7 gene in other mammalian species. The probes can either be positive strand or anti-sense strands, and they can be comprised of DNA or RNA.

15 The present invention is further comprised of polypeptides comprised of a subsequence of SEQ ID NO:2. The subsequence is comprised of from 20 to 552 amino acids residues from SEQ ID NO:2 which are antigenic and against  
20 which a warm blooded animal is able to produce antibodies against when the polypeptide is injected into the animal and said antibodies are able to bind to the polypeptide of SEQ ID NO: 2. Preferably, the subsequence is a subsequence of SEQ ID NO: 13. Preferably, the subsequence contains at  
25 least 50 amino acids from SEQ ID NO: 2.

These and other aspects of the invention will become evident upon reference to the following detailed description and the attached drawing.

30

#### DETAILED DESCRIPTION OF THE INVENTION

The term "allelic variant" is used herein to  
35 denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation

arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems.

"Operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural



sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules.

5 The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

10

The term "receptor" is used herein to denote a cell-associated protein, or a polypeptide subunit of such a protein, that binds to a bioactive molecule (the "ligand") and mediates the effect of the ligand on the  
15 cell. Binding of ligand to receptor results in a conformational change in the receptor (and, in some cases, receptor multimerization, i.e., association of identical or different receptor subunits) that causes interactions between the effector domain(s) and other molecule(s) in  
20 the cell. These interactions in turn lead to alterations in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, cell proliferation, increases in cyclic AMP production,  
25 mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. The term "receptor polypeptide" is used to denote complete receptor polypeptide chains and portions thereof, including  
30 isolated functional domains (e.g., ligand-binding domains).

A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide") that,  
35 as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which

it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

5           A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors can comprise additional amino acid residues,  
10 such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are  
15 produced by proteolysis or translated from alternatively spliced mRNAs. Receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal  
20 transduction, respectively.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a protein having the structure of a cytokine receptor, including the  
25 conserved WSXWS motif (SEQ ID NO:10). Analysis of the tissue distribution of the mRNA corresponding to this novel DNA showed that mRNA level was highest in pancreas, prostate, kidney and adrenal cortex followed by lower levels in testis, stomach, adrenal medulla and thymus. The  
30 receptor has been designated "Zcytor7".

Cytokine receptors subunits are characterized by a multi-domain structure comprising a ligand-binding  
35 domain and an effector domain that is typically involved in signal transduction. Multimeric cytokine receptors

include homodimers (e.g., PDGF receptor  $\alpha\alpha$  and  $\beta\beta$  isoforms, erythropoietin receptor, MPL [thrombopoietin receptor], and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor  $\alpha\beta$  isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal transduction domain, is a component of IL-3 and GM-CSF receptors. Many cytokine receptors can be placed into one of four related families on the basis of their structures and functions. Class I hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS motif (SEQ ID NO:10). Additional domains, including protein kinase domains; fibronectin type III domains; and immunoglobulin domains, which are characterized by disulfide-bonded loops, are present in certain hematopoietic receptors. Cytokine receptor structure has been reviewed by Urdal, *Ann. Reports Med. Chem.* 26:221-228 (1991) and Cosman, *Cytokine* 5:95-106 (1993). It is generally believed that under selective pressure for organisms to acquire new biological functions, new receptor family members arose from duplication of existing receptor genes leading to the existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation and identification of additional family members.

Cell-surface cytokine receptors are further characterized by the presence of additional domains. These receptors are anchored in the cell membrane by a transmembrane domain characterized by a sequence of

hydrophobic amino acid residues (typically about 21-25 residues), which is commonly flanked by positively charged residues (Lys or Arg). On the opposite end of the protein from the extracellular domain and separated from it by the transmembrane domain is an intracellular domain.

The novel receptor of the present invention, Zcytor7, is a class II cytokine receptor. These receptors usually bind to four-helix-bundle cytokines. Interleukin-10 and the interferons have receptors in this class (e.g., interferon-gamma alpha and beta chains and the interferon-alpha/beta receptor alpha and beta chains). Class II cytokine receptors are characterized by the presence of one or more cytokine receptor modules (CRM) in their extracellular domains. The CRMs of class II cytokine receptors are somewhat different than the better known CRMs of class I cytokine receptors. While the class II CRMs contain two type-III fibronectin-like domains, they differ in organization. In particular, they contain two WSXWS (SEQ ID NO: 10) motifs, one in each fibronectin III-like domain. These WSXWS (SEQ ID NO: 10) motifs, however, are less conserved than those found in class I CRMs.

Zcytor7, like all known class II receptors except interferon-alpha/beta receptor alpha chain, has only a single class II CRM in its extracellular domain. Zcytor7 appears to be a receptor for a helical cytokine of the interferon/IL-10 class. Using the Zcytor7 receptor we can identify ligands and additional compounds which would be of significant therapeutic value. Furthermore, the extracellular portion of Zcytor7 extending from residue 30, a valine, through residue 250 of SEQ ID NO: 2 can be expressed and used as a soluble receptor to down-regulate the effects of the ligand of Zcytor7.

As was stated above, Zcytor7 was initially identified by the overall homology to CRF2-4, an orphan Class II cytokine receptor. See Lutfalla G. et al. *Genomics*, 16: 366-373 (1993). Analysis of a human cDNA clone encoding Zcytor7 (SEQ ID NO:1) revealed an open reading frame encoding 553 amino acids (SEQ ID NO:2) comprising an extracellular ligand-binding domain of approximately 221 amino acid residues (residues 30-250 of SEQ ID NO:2), a transmembrane domain of approximately 24 amino acid residues (residues 251-274 of SEQ ID NO:2), and an intracellular domain of approximately 279 amino acid residues (residues 275-553 of SEQ ID NO:2). Those skilled in the art will recognize that these domain boundaries are approximate and are based on alignments with known proteins and predictions of protein folding. Deletion of residues from the ends of the domains is possible.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1 or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is at least about 0.02 M at pH 7 and the temperature is at least about 60°C. As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from pancreas or prostate tissues although cDNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction

followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., *Biochemistry* 18:52-94 (1979). Poly (A)<sup>+</sup> RNA is prepared from total RNA using the method of Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412 (1972).

5 Complementary DNA (cDNA) is prepared from poly(A)<sup>+</sup> RNA using known methods. Polynucleotides encoding Zcytor7 polypeptides are then identified and isolated by, for example, hybridization or PCR.

10 Those skilled in the art will recognize that the sequences disclosed in SEQ ID NOS:1 and 2 represent single alleles of the human Zcytor7 receptor. Allelic variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals according to  
15 standard procedures.

The present invention further provides counterpart receptors and polynucleotides from other species ("species orthologs"). Of particular interest are  
20 Zcytor7 receptors from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate receptors. Species orthologs of the human Zcytor7 receptor can be cloned using information and compositions provided by the present invention in  
25 combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the receptor. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed  
30 herein. A library is then prepared from mRNA of a positive tissue or cell line. A receptor-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed  
35 sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No.

4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected  
5 with an antibody to the receptor. Similar techniques can also be applied to the isolation of genomic clones.

The present invention also provides isolated receptor polypeptides that are substantially homologous to  
10 the receptor polypeptide of SEQ ID NO: 2. By "isolated" is meant a protein or polypeptide that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other  
15 polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. The term "substantially homologous" is used herein to denote polypeptides having  
20 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2,. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2. Percent sequence identity is determined by  
25 conventional methods. See, for example, Altschul et al., *Bull. Math. Bio.* 48: 603-616 (1986) and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty  
30 of 10, a gap extension penalty of 1, and the "blossom 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 1 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

5



Table 1

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
5	4																			
	R -1	5																		
	N -2	0	6																	
	D -2	-2	1	6																
	C 0	-3	-3	-3	9															
	Q -1	1	0	0	-3	5														
10	E -1	0	0	2	-4	2	5													
	G 0	-2	0	-1	-3	-2	-2	6												
	H -2	0	1	-1	-3	0	0	-2	8											
	I -1	-3	-3	-3	-1	-3	-3	-4	-3	4										
	L -1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
15	K -1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
	M -1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
	F -2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
	P -1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-1	-2	-4	7						
	S 1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
20	T 0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
	W -3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
	Y -2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
	V 0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

5 Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 2) and other substitutions  
10 that do not significantly affect the folding or activity of the protein or polypeptide. Also claimed are deletions of SEQ ID NO:2, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker  
15 peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A, Nilsson et al., *EMBO J.* 4:1075 (1985); Nilsson et al., *Methods Enzymol.* 198:3 (1991), glutathione S transferase, Smith and Johnson, *Gene* 67:31 (1988), or other antigenic epitope or binding  
20 domain. See, in general Ford et al., *Protein Expression and Purification* 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

25

Table 2Conservative amino acid substitutions

30	Basic:	arginine
		lysine
		histidine
	Acidic:	glutamic acid
		aspartic acid
	Polar:	glutamine
		asparagine

TABLE 2, continued

	Hydrophobic:	leucine
		isoleucine
		valine
5		
	Aromatic:	phenylalanine
		tryptophan
		tyrosine
	Small:	glycine
10		alanine
		serine
		threonine
		methionine
15	Essential amino acids in the receptor	
	polypeptides of the present invention can be identified	
	according to procedures known in the art, such as site-	
	directed mutagenesis or alanine-scanning mutagenesis,	
	Cunningham and Wells, <i>Science</i> 244, 1081-1085 (1989); Bass	
20	<i>et al.</i> , <i>Proc. Natl. Acad. Sci. USA</i> 88:4498-4502 (1991).	
	In the latter technique, single alanine mutations are	
	introduced at every residue in the molecule, and the	
	resultant mutant molecules are tested for biological	
	activity (e.g., ligand binding and signal transduction) to	
25	identify amino acid residues that are critical to the	
	activity of the molecule. Sites of ligand-receptor	
	interaction can also be determined by analysis of crystal	
	structure as determined by such techniques as nuclear	
	magnetic resonance, crystallography or photoaffinity	
30	labeling. See, for example, de Vos <i>et al.</i> , <i>Science</i>	
	255:306-312 (1992); Smith <i>et al.</i> , <i>J. Mol. Biol.</i> 224:899-	
	904 (1992); Wlodaver <i>et al.</i> , <i>FEBS Lett.</i> 309:59-64 (1992).	
	The identities of essential amino acids can also be	
	inferred from analysis of homologies with related	
35	receptors.	

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer, *Science* 241:53-57 (1988) or Bowie and Sauer, *Proc. Natl. Acad. Sci. USA* 86:2152-2156 (1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., *Biochem.* 30:10832-10837 (1991); Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis, Derbyshire et al., *Gene* 46:145 (1986); Ner et al., *DNA* 7:127 (1988).

Mutagenesis methods as disclosed above can be combined with high-throughput screening methods to detect activity of cloned, mutagenized receptors in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active receptors or portions thereof (e.g., ligand-binding fragments) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

30

Using the methods discussed above, one of ordinary skill in the art can prepare a variety of polypeptides that are substantially homologous to residues 30 to 250 of SEQ ID NO:2 or allelic variants thereof and retain the ligand-binding properties of the wild-type receptor. Such polypeptides may include additional amino

35

acids from an extracellular ligand-binding domain of a Zcytor7 receptor as well as part or all of the transmembrane and intracellular domains. Such polypeptides may also include additional polypeptide  
5 segments as generally disclosed above.

Polynucleotides, generally a cDNA sequence, of the present invention encode the above-described polypeptides. A cDNA sequence which encodes a polypeptide  
10 of the present invention is comprised of a series of codons, each amino acid residue of the polypeptide being encoded by a codon and each codon being comprised of three nucleotides. The amino acid residues are encoded their respective codons as follows.

15

Alanine (Ala) is encoded by GCA, GCC, GCG or GCT;

20

Cysteine (Cys) is encoded by TGC or TGT;  
Aspartic acid (Asp) is encoded by GAC or GAT;  
Glutamic acid (Glu) is encoded by GAA or GAG;  
Phenylalanine (Phe) is encoded by TTC or TTT;  
Glycine (Gly) is encoded by GGA, GGC, GGG or GGT;

25

Histidine (His) is encoded by CAC or CAT;  
Isoleucine (Ile) is encoded by ATA, ATC or ATT;  
Lysine (Lys) is encoded by AAA, or AAG;  
Leucine (Leu) is encoded by TTA, TTG, CTA, CTC, CTG or CTT;

30

Methionine (Met) is encoded by ATG;  
Asparagine (Asn) is encoded by AAC or AAT;  
Proline (Pro) is encoded by CCA, CCC, CCG or CCT;

35

Glutamine (Gln) is encoded by CAA or CAG;  
Arginine (Arg) is encoded by AGA, AGG, CGA, CGC, CGG or CGT;

Serine (Ser) is encoded by AGC, AGT, TCA, TCC, TCG or TCT;

Threonine (Thr) is encoded by ACA, ACC, ACG or ACT;

5 Valine (Val) is encoded by GTA, GTC, GTG or GTT;  
Tryptophan (Trp) is encoded by TGG; and  
Tyrosine (Tyr) is encoded by TAC or TAT.

It is to be recognized that according to the  
10 present invention, when a cDNA is claimed as described  
above, it is understood that what is claimed are both the  
sense strand, the anti-sense strand, and the DNA as  
double-stranded having both the sense and anti-sense  
strand annealed together by their respective hydrogen  
15 bonds. Also claimed is the messenger RNA (mRNA) encodes  
the polypeptides of the present invention, and which mRNA  
is encoded by the above the above-described cDNA. A  
messenger RNA (mRNA) will encode a polypeptide using the  
same codons as those defined above, with the exception  
20 that each thymine nucleotide (T) is replaced by a uracil  
nucleotide (U).

The receptor polypeptides of the present  
invention, including full-length receptors, receptor  
25 fragments (e.g. ligand-binding fragments), and fusion  
polypeptides can be produced in genetically engineered  
host cells according to conventional techniques. Suitable  
host cells are those cell types that can be transformed or  
transfected with exogenous DNA and grown in culture, and  
30 include bacteria, fungal cells, and cultured higher  
eukaryotic cells. Eukaryotic cells, particularly cultured  
cells of multicellular organisms, are preferred.  
Techniques for manipulating cloned DNA molecules and  
introducing exogenous DNA into a variety of host cells are  
35 disclosed by Sambrook et al., *Molecular Cloning: A  
Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory

Press, Cold Spring Harbor, NY (1989), and Ausubel et al.,  
*ibid.*

The polynucleotides of the present invention can  
5 be synthesized using DNA synthesizers. Currently the  
method of choice is the phosphoramidite method.

An initial set of overlapping, complementary  
oligonucleotides, each of which is between 20 to 60  
10 nucleotides long is first made. Each internal section of  
the gene has complementary 3' and 5' terminal extensions  
that are designed to base pair precisely with an adjacent  
section. Thus, after the gene is assembled, the only  
remaining requirement to complete the process is sealing  
15 the nicks along the backbones of the two strands with T4  
DNA ligase. Furthermore, terminal sequences that  
facilitate insertion into a restriction endonuclease sites  
of a cloning vector and other sequences should also be  
added that contain signals for the proper initiation and  
20 termination of transcription and translation.

An alternative way to prepare a full-size gene  
is to synthesize a specified set of overlapping  
oligonucleotides (40 to 100 nucleotides). The duplex is  
25 completed and the gaps filled by enzymatic DNA synthesis  
with *E. coli* DNA polymerase I. This enzyme uses the 3'-  
hydroxyl groups as replication initiation points and the  
single-stranded regions as templates. After the enzymatic  
synthesis is completed, the nicks are sealed with T4 DNA  
30 ligase. For larger genes ( $\geq 1,000$  base pairs), the  
complete gene sequence is usually assembled from double-  
stranded fragments that are each put together by joining  
four to six overlapping oligonucleotides (20 to 60 bp  
each). If there is a sufficient amount of the double-  
35 stranded fragments after each synthesis and annealing  
step, they are simply joined to one another. Otherwise,

each fragment is cloned into a vector to amplify the amount of DNA available. In both cases, the double-stranded constructs are sequentially linked to one another to form the entire gene sequence. See Glick, Bernard R. and Jack J. Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA*, (ASM Press, Washington, D.C. 1994), Itakura, K. et al. Synthesis and use of synthetic oligonucleotides. *Annu. Rev. Biochem.* 53 : 323-356 (1984), and Climie, S. et al. Chemical synthesis of the thymidylate synthase gene. *Proc. Natl. Acad. Sci. USA* 87 :633-637 (1990).

A DNA sequence encoding a Zcytor7 receptor polypeptide can then be operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a Zcytor7 receptor polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the receptor, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is joined to the Zcytor7 DNA sequence in the correct



reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, 5 e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Cultured mammalian cells are preferred hosts within the present invention. Methods for introducing 10 exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection, Wigler et al., *Cell* 14:725 (1978); Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981; Graham and Van der Eb, *Virology* 52:456 (1973), electroporation, Neumann et al., *EMBO J.* 1:841-845 15 (1982), DEAE-dextran mediated transfection, Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY (1987), and liposome-mediated transfection, Hawley-Nelson et al., *Focus* 15:73 (1993); Ciccarone et al., *Focus* 15:80 (1993). The production of 20 recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian 25 cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72 (1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable 30 cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. 35 Other suitable promoters include those from

metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for  
5 cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as  
10 "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the  
15 gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high  
20 levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can  
25 also be used.

Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of  
30 foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222; Bang et al., U.S. Patent No. 4,775,624; and WIPO publication WO 94/06463. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al.,  
35 *J. Biosci. (Bangalore)* 11:47-58 (1987).

Fungal cells, including yeast cells, and particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing receptor fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.* 132:3459-3465 (1986) and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

Within one aspect of the present invention, a novel receptor is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand, as well as agonists and antagonists of the natural ligand. To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (e.g., a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems.

Mammalian cells suitable for use in expressing Zcytor7 receptors and transducing a receptor-mediated signal include cells that express other receptor subunits which may form a functional complex with Zcytor7. These subunits may include those of the interferon receptor family or of other class II or class I cytokine receptors. It is also preferred to use a cell from the same species as the receptor to be expressed. Within a preferred

embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for its proliferation. Preferred cell lines of this type are the human TF-1 cell line (ATCC number CRL-2003) and the AML-  
5 193 cell line (ATCC number CRL-9589), which are GM-CSF-dependent human leukemic cell lines. In the alternative, suitable host cells can be engineered to produce the necessary receptor subunit or other cellular component needed for the desired cellular response. For example,  
10 the murine cell line BaF3, Palacios and Steinmetz, *Cell* 41: 727-734 (1985); Mathey-Prevot et al., *Mol. Cell. Biol.* 6: 4133-4135 (1986) or a baby hamster kidney (BHK) cell line can be transfected to express the necessary  $\beta$  subunit (also known as KH97) as well as a Zcytor7 receptor. The  
15 latter approach is advantageous because cell lines can be engineered to express receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. In the alternative, species orthologs of the human receptor cDNA can be cloned and  
20 used within cell lines from the same species, such as a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as GM-CSF, can thus be engineered to become dependent upon a Zcytor7 ligand.

25

Cells expressing functional receptor are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the detection of a biological response in a target cell. One such assay  
30 is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-  
35 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Mosman, *J. Immunol. Meth.* 65: 55-63 (1983). An

alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred promoter element in this regard is a serum response element, or SRE (see, e.g., Shaw et al., *Cell* 56:563-572 (1989)). A preferred such reporter gene is a luciferase gene, de Wet et al., *Mol. Cell. Biol.* 7:725 (1987).

Expression of the luciferase gene is detected by luminescence using methods known in the art, e.g., Baumgartner et al., *J. Biol. Chem.* 269:29094-29101 (1994); Schenborn and Goiffin, *Promega Notes* 41:11 (1993). Luciferase activity assay kits are commercially available from, for example, Promega Corp., Madison, WI. Target cell lines of this type can be used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell-conditioned media samples can be assayed on a target cell to identify cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian expression vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools, re-transfection, subculturing, and re-assay of positive cells to isolate a cloned cDNA encoding the ligand.

A natural ligand for the Zcytor7 receptor can also be identified by mutagenizing a cell line expressing the receptor and culturing it under conditions that select for autocrine growth. See WIPO publication WO 95/21930. Within a typical procedure, BaF3 cells expressing Zcytor7 and the necessary additional subunits are mutagenized, such as with 2-ethylmethanesulfonate (EMS). The cells are then allowed to recover in the presence of IL-3, then

transferred to a culture medium lacking IL-3 and IL-4. Surviving cells are screened for the production of a Zcytor7 ligand, such as by adding soluble receptor to the culture medium or by assaying conditioned media on wild-  
5 type BaF3 cells and BaF3 cells expressing the receptor.

An additional screening approach provided by the present invention includes the use of hybrid receptor polypeptides. These hybrid polypeptides fall into two  
10 general classes. Within the first class, the intracellular domain of Z-Cytor7, comprising approximately residues 275 to 553 of SEQ ID NO:2, is joined to the ligand-binding domain of a second receptor. It is preferred that the second receptor be a hematopoietic  
15 cytokine receptor, such as mpl receptor, Souyri et al., Cell 63: 1137-1147 (1990). The hybrid receptor will further comprise a transmembrane domain, which may be derived from either receptor. A DNA construct encoding the hybrid receptor is then inserted into a host cell.  
20 Cells expressing the hybrid receptor are cultured in the presence of a ligand for the binding domain and assayed for a response. This system provides a means for analyzing signal transduction mediated by Zcytor7 while using readily available ligands. This system can also be  
25 used to determine if particular cell lines are capable of responding to signals transduced by Zcytor7. A second class of hybrid receptor polypeptides comprise the extracellular (ligand-binding) domain of Zcytor7 (approximately residues 30 to 250 of SEQ ID NO:2) with an  
30 intracellular domain of a second receptor, preferably a hematopoietic cytokine receptor, and a transmembrane domain. Hybrid receptors of this second class are expressed in cells known to be capable of responding to signals transduced by the second receptor. Together,  
35 these two classes of hybrid receptors enable the

identification of a responsive cell type for the development of an assay for detecting a Zcytor7 ligand.

Cells found to express the ligand are then used  
5 to prepare a cDNA library from which the ligand-encoding cDNA can be isolated as disclosed above. The present invention thus provides, in addition to novel receptor polypeptides, methods for cloning polypeptide ligands for the receptors.

10

The tissue specificity of Zcytor7 expression suggests a role in the development of the kidney, pancreas, prostate or nervous tissues. In view of the tissue specificity observed for this receptor, agonists  
15 (including the natural ligand) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as receptor agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For example,  
20 agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists may be useful in specifically promoting the growth and/or development of  
25 nervous, pancreatic or prostate-derived cells in culture. Antagonists are useful as research reagents for characterizing sites of ligand-receptor interaction. *In vivo*, receptor agonists or antagonists may find application in the treatment of renal, neural, pancreatic  
30 or prostate diseases.

Zcytor7 may also be used within diagnostic systems for the detection of circulating levels of ligand. Within a related embodiment, antibodies or other agents  
35 that specifically bind to Zcytor7 can be used to detect circulating receptor polypeptides. Elevated or depressed



levels of ligand or receptor polypeptides may be indicative of pathological conditions, including cancer.

Zcytor7 receptor polypeptides can be prepared by  
5 expressing a truncated DNA encoding the extracellular  
domain, for example, a polypeptide which contains residues  
30 through 250 of a human Zcytor7 receptor (SEQ ID NO:2)  
or the corresponding region of a non-human receptor. It  
is preferred that the extracellular domain polypeptides be  
10 prepared in a form substantially free of transmembrane and  
intracellular polypeptide segments. For example, the C-  
terminus of the receptor polypeptide may be at residue 250  
of SEQ ID NO:2 or the corresponding region of an allelic  
variant or a non-human receptor. To direct the export of  
15 the receptor domain from the host cell, the receptor DNA  
is linked to a second DNA segment encoding a secretory  
peptide, such as a t-PA secretory peptide. To facilitate  
purification of the secreted receptor domain, a C-terminal  
extension, such as a poly-histidine tag, substance P, Flag  
20 <sup>TM</sup> peptide, Hopp et al., *Biotechnology* 6:1204-1210 (1988),  
available from Eastman Kodak Co., New Haven, CT or another  
polypeptide or protein for which an antibody or other  
specific binding agent is available, can be fused to the  
receptor polypeptide.

25

In an alternative approach, a receptor  
extracellular domain can be expressed as a fusion with  
immunoglobulin heavy chain constant regions, typically an  
F<sub>C</sub> fragment, which contains two constant region domains  
30 and a hinge region but lacks the variable region. Such  
fusions are typically secreted as multimeric molecules  
wherein the F<sub>C</sub> portions are disulfide bonded to each other  
and two receptor polypeptides are arrayed in closed  
proximity to each other. Fusions of this type can be used  
35 to affinity purify the cognate ligand from solution, as an  
in vitro assay tool, to block signals in vitro by

specifically titrating out ligand, and as antagonists *in vivo* by administering them parenterally to bind circulating ligand and clear it from the circulation. To purify ligand, a Zcytor7-Ig chimera is added to a sample  
5 containing the ligand (e.g., cell-conditioned culture media or tissue extracts) under conditions that facilitate receptor-ligand binding (typically near-physiological temperature, pH, and ionic strength). The chimera-ligand complex is then separated by the mixture using protein A,  
10 which is immobilized on a solid support (e.g., insoluble resin beads). The ligand is then eluted using conventional chemical techniques, such as with a salt or pH gradient. In the alternative, the chimera itself can be bound to a solid support, with binding and elution  
15 carried out as above. Chimeras with high binding affinity are administered parenterally (e.g., by intramuscular, subcutaneous or intravenous injection). Circulating molecules bind ligand and are cleared from circulation by normal physiological processes. For use in assays, the  
20 chimeras are bound to a support via the F<sub>C</sub> region and used in an ELISA format.

A preferred assay system employing a ligand-binding receptor fragment uses a commercially available  
25 biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ), wherein the receptor fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, *J. Immunol. Methods* 145:229-240 1991 and Cunningham and Wells, *J. Mol.*  
30 *Biol.* 234:554-563 (1993). A receptor fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If ligand is present in the sample, it will bind to the  
35 immobilized receptor polypeptide, causing a change in the refractive index of the medium, which is detected as a

change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

5

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity, [see, Scatchard, Ann. NY Acad. Sci. 51: 660-672 (1949) and calorimetric assays, Cunningham et al., *Science* 253:545-548 (1991); Cunningham et al., *Science* 254:821-825 (1991).

A receptor ligand-binding polypeptide can also be used for purification of ligand. The receptor polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting media will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration or pH to disrupt ligand-receptor binding.

Zcytor7 polypeptides can also be used to prepare antibodies that specifically bind to Zcytor7 epitopes, peptides or polypeptides. The Zcytor7 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. Suitable antigens would be a polypeptide comprised of a

subsequence of the Zcytor7 polypeptide against which antibodies can be produced which bind to the Zcytor7 polypeptide either in its native or denatured state. The Antibodies generated from this immune response can be  
5 isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, *Current Protocols in Immunology*, Cooligan, et al. (eds.), (National Institutes of Health, John Wiley and Sons, Inc.,  
10 1995); Sambrook et al., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, (CRC Press, Inc., Boca Raton, FL, 1982).

15

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice,  
20 and rats with a Zcytor7 polypeptide or a fragment thereof. The immunogenicity of a Zcytor7 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also  
25 include fusion polypeptides, such as fusions of Zcytor7 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may  
30 be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes  
35 polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding

fragments, such as F(ab')<sub>2</sub> and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced.

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to Zcytor7 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled Zcytor7 protein or peptide). Genes encoding polypeptides having potential Zcytor7 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide

display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide  
5 display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide  
10 display libraries can be screened using the Zcytor7 sequences disclosed herein to identify proteins which bind to Zcytor7. These "binding proteins" which interact with Zcytor7 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification;  
15 they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for  
20 diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble polypeptides as marker of underlying pathology or disease. These binding proteins can also act as Zcytor7 "antagonists" to block Zcytor7 binding and signal  
25 transduction *in vitro* and *in vivo*. These anti-Zcytor7 binding proteins would be useful for inhibiting ???.

Antibodies are determined to be specifically binding if: 1) they exhibit a threshold level of binding  
30 activity, and/or 2) they do not significantly cross-react with related polypeptide molecules. First, antibodies herein specifically bind if they bind to a Zcytor7 polypeptide, peptide or epitope with a binding affinity ( $K_a$ ) of  $10^6 \text{ M}^{-1}$  or greater, preferably  $10^7 \text{ M}^{-1}$  or greater,  
35 more preferably  $10^8 \text{ M}^{-1}$  or greater, and most preferably

$10^9 \text{ M}^{-1}$  or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis, Scatchard, G., *Ann. NY Acad. Sci.* 51: 660-672 (1949).

5

Second, antibodies are determined to specifically bind if they do not significantly cross-react with related polypeptides. Antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect Zcytor7 but not known related polypeptides using a standard Western blot analysis (Ausubel et al., *ibid.*). Examples of known related polypeptides are orthologs, proteins from the same species that are members of a protein family (e.g. IL-16), Zcytor7 polypeptides, and non-human Zcytor7. Moreover, antibodies may be "screened against" known related polypeptides to isolate a population that specifically binds to the inventive polypeptides. For example, antibodies raised to Zcytor7 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to Zcytor7 will flow through the matrix under the proper buffer conditions. Such screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to closely related polypeptides, *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), (Cold Spring Harbor Laboratory Press, 1988); *Current Protocols in Immunology*, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art. See, *Fundamental Immunology*, Paul (eds.), (Raven Press, 1993); Getzoff et al., *Adv. in Immunol.* 43: 1-98 (1988); *Monoclonal Antibodies: Principles and Practice*, Goding, J.W. (eds.), (Academic Press Ltd., 1996); Benjamin et al., *Ann. Rev. Immunol.* 2: 67-101 (1984).

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A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Zcytor7 proteins or peptides.

5 Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), (Cold Spring Harbor Laboratory Press, 1988). Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked  
10 immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant Zcytor7 protein or polypeptide.

15

Antibodies to Zcytor7 may be used for tagging cells that express Zcytor7; for isolating Zcytor7 by affinity purification; for diagnostic assays for determining circulating levels of Zcytor7 polypeptides;  
20 for detecting or quantitating soluble Zcytor7 as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block Zcytor7 \$\$ *in vitro* and *in vivo*. Suitable direct tags or labels include  
25 radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-  
30 complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to Zcytor7 or fragments thereof may  
35 be used *in vitro* to detect denatured Zcytor7 or fragments



thereof in assays, for example, Western Blots or other assays known in the art.

Anti-idiotypic antibodies which bind to the antigenic binding site of antibodies to Zcytor7 are also considered part of the present invention. The antigenic binding region of the anti-idiotypic antibody thus will mimic the ligand binding region of Zcytor7. An anti-idiotypic antibody thus could be used to screen for possible ligands of the Zcytor7 receptor. Thus neutralizing antibodies to Zcytor7 can be used to produce anti-idiotypic antibodies by methods well known in the art as is described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, (CRC Press, Inc., Boca Raton, FL, 1982).

The present invention also provides reagents which will find use in diagnostic applications. For example, the Zcytor7 gene, a probe comprising Zcytor7 DNA or RNA or a subsequence thereof can be used to determine if the Zcytor7 gene is present on chromosome 6 or if a mutation has occurred. Detectable chromosomal aberrations at the Zcytor7 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., *ibid.*; Ausubel et. al., *ibid.*; Marian, Chest 108:255-65, 1995).

Transgenic mice, engineered to express the Zcytor7 gene, and mice that exhibit a complete absence of Zcytor7 gene function, referred to as "knockout mice", Snouwaert et al., *Science* 257:1083 (1992), may also be  
5 generated, Lowell et al., *Nature* 366:740-42 (1993). These mice may be employed to study the Zcytor7 gene and the protein encoded thereby in an *in vivo* system.

#### CHROMOSOMAL LOCALIZATION:

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Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes, Cox et al., *Science* 250:245-50, (1990). Partial or full  
15 knowledge of a gene's sequence allows one to design PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Radiation hybrid mapping panels are commercially available which cover the entire human genome, such as the Stanford G3 RH Panel and the  
20 GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL). These panels enable rapid, PCR-based chromosomal localizations and ordering of genes, sequence-tagged sites (STSs), and other nonpolymorphic and polymorphic markers within a region of interest. This  
25 includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an  
30 existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model  
35 organisms, such as mouse, which may aid in determining what function a particular gene might have.

Sequence tagged sites (STSs) can also be used independently for chromosomal localization. An STS is a DNA sequence that is unique in the human genome and can be used as a reference point for a particular chromosome or region of a chromosome. An STS is defined by a pair of oligonucleotide primers that are used in a polymerase chain reaction to specifically detect this site in the presence of all other genomic sequences. Since STSs are based solely on DNA sequence they can be completely described within an electronic database, for example, Database of Sequence Tagged Sites (dbSTS), GenBank, (National Center for Biological Information, National Institutes of Health, Bethesda, MD <http://www.ncbi.nlm.nih.gov>), and can be searched with a gene sequence of interest for the mapping data contained within these short genomic landmark STS sequences.

Zcytor-7 maps 795.76 cR from the top of the human chromosome 6 linkage group on the WICGR radiation hybrid map. Relative to the centromere, its nearest proximal marker was CHLC.GATA32B03 and its nearest distal marker was SGC32063. The use of surrounding markers also helped position zcytor-7 in the 6q22-q23 region on the CHLC chromosome 6 version v8c7 integrated marker map. The locus where Zcytor7 maps onto chromosome 6 is a common breakpoint area in ALL(acute lymphoblastic leukemia) and NHL(non-Hodgkin lymphoma) as well as in AML(acute myelogenous leukemia) and CML(chronic myeloid leukemia). It is interesting to note that the MYB (avian myeloblastosis viral oncogene homolog) gene, which encodes proteins critical for hematopoietic cell proliferation and development, appears to be less than 800 kB from Zcytor7. The 6q- deletion breakpoints occur slightly distal to the

MYB gene and although the neoplasms show high levels of MYB mRNA, the gene itself appears to be intact.

Thus Zcytor7 could be used to generate a probe that  
5 could allow detection of an aberration of the Zctyor7 gene  
in the 6q chromosome which may indicate the presence of a  
cancerous cell such as leukemic cells which may still be  
present in after chemical or radiation therapy. If the  
Zcytor7 gene is deleted by the chromosomal abnormality,  
10 only one copy can be used to determine whether one or two  
copies of the gene are present per nucleus, thus  
indicating the percentage cancerous cells might be present  
relative to normal cells. For further discussions on  
developing polynucleotide probes and hybridization see  
15 *Current Protocols in Molecular Biology* Ausubel, F. et al.  
Eds. (John Wiley & Sons Inc. 1991).

#### *Pharmaceutical Compositions*

Pharmaceutical compositions can be formulated  
20 which contain the soluble receptor, antibody or anti-  
idiotypic antibodies of the present invention. Generally  
included in such protein therapeutic compositions are  
buffers; surface adsorption inhibitors such as surfactants  
and polyols; and isotonic amounts of a physiologically  
25 acceptable salt. The composition may be formulated as an  
aqueous solution or a lyophilized powder. The latter is  
reconstituted prior to use with a pharmaceutically  
acceptable diluent such as sterile water for injection.

30 Examples of buffers which can be used for the above-  
described pharmaceutical compositions include low ionic  
strength, physiologically acceptable buffers that are  
effective within the pH range of 5.0 - 7.0. Such buffers  
include phosphate, acetate, citrate, succinate and  
35 histidine buffers.

Examples of surface adsorption inhibitors which can be used in the above-described pharmaceutical compositions include non-ionic surfactants and polyols. Non-ionic surfactants include polyoxyethylene sorbitan fatty acid esters, such as polysorbate 20 (polyoxyethylene sorbitan monolaurate), and the like. Other non-ionic surfactants useful in this regard include polyethylene oxides; sorbitan esters; polyoxyethylene alkyl ethers; and glycerides of fatty acid, including glyceryl monooleate and glyceryl monostearate. Polyols which can be used include polyethylene glycol, e.g. PEG 3350, mannitol, xylitol, sorbitol, inositol, and glycerol. In general, the surface adsorption inhibitor will be included within the composition at a concentration from 0.001% to 5%.

15

Physiologically acceptable salts are generally included in a protein therapeutic composition generally in an amount isotonic to human blood. Preferred salts in this regard include chloride salts such as NaCl, KCl, CaCl<sub>2</sub> and MgCl<sub>2</sub>.

20

Albumin may also be included in the above-described pharmaceutical compositions. Human serum albumin is preferred for inclusion in pharmaceutical compositions intended for human use. Albumin is useful as an excipient in lyophilized compositions and acts as a stabilizer when included at a concentration of 0.1 - 1.0%. Albumin may useful as a surface adsorption inhibitor.

30

One or more preservatives may also be included in the pharmaceutical compositions of the present invention. Common preservative include methylparaben, propylparaben, benzyl alcohol, m-cresol, ethylmercurithiosalicylate, phenol, thimerosal and the like. Methods of formulation of pharmaceutical compositions are well known in the art and are disclosed, for example, in *Remington's Pharmaceutical*

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Sciences, Gennaro, ed., (Mack Publishing Co., Easton, PA, 1996)

### *Dosages*

5

Therapeutic doses of the protein compositions of the present invention will generally be in the range of 0.1 to 100 µg/kg of patient per day with the exact dose determined by the clinician.

10

The invention is further illustrated by the following non-limiting examples.

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#### Example 1.

##### Cloning of Zcytor7

Expressed sequence tag SEQ ID NO:3 was identified. A  
20 cDNA clone was discovered which contained a partial sequence. The cDNA sequence was determined to be 1231 bp in length, but was not a full length sequence.

A human testis cDNA template was made using a  
25 MARATHON™ cDNA Amplification Kit (Clontech Laboratories, Inc., Palo Alto, CA) according to the supplier's instructions. A 5' RACE reaction was used to obtain a full-length cDNA. The RACE reaction was carried out in two reactions employing two sets of primers. Reaction I (outer  
30 nest), using primers SEQ ID NO:4 and AP-1 (SEQ ID NO: 5) (Clontech Laboratories) was run for 35 cycles at 98°C for 20 seconds, 45°C for 20 seconds; 68° for 4 minutes and a final extension time of 10 minutes at 68°C. One µl of a 1:100 dilution of the reaction product was used as a  
35 template in reaction II (inner nest). Primers were ZC11,108 (SEQ ID NO:6 ) and AP-2 (SEQ ID NO:7 ) (Clontech Laboratories). The reaction was run at 98°C for 30 seconds, and 30 cycles each cycle being comprised of 98°C for 28

seconds; 43°C for 20 seconds; and 68°C for 3.5 minutes with a final extension at 68°C for 10 minutes.

The product of the inner nest RACE reaction was  
5 subcloned using a PCR-SCRIPT™ kit (Stratagene Cloning Systems, La Jolla, CA) to prepare the plasmid pSLR7-1. Sequence analysis of this plasmid indicated that the 5'RACE-generated sequence extended the sequence of pSL7139 by 555 bp.

10

Full-length cDNA was obtained by screening a λZAP® II human testis cDNA library using a probe that was generated by PCR primers ZC11,526 (SEQ ID NO:9) and ZC11,108 (SEQ ID NO:6) and pSLR7-1 as template and then re-amplified. The  
15 resulting probe was purified through recovery from low-melt agarose gel electrophoresis and was labeled with <sup>32</sup>P-α-dCTP using a MEGAPRIME™ labeling kit (Amersham Corp., Arlington, Heights, IL). The labeled probe was purified on a push column (NUCTRAP® probe purification column;  
20 Stratagene Cloning Systems).

The first strand cDNA reaction contained 15 µl of human testis twice poly d(T)-selected poly (A)<sup>+</sup> mRNA (Clontech Laboratories) at a concentration of 1.0 µg/µl,  
25 and 3 µl of 20 pmole/µl first strand primer ZC6091 (SEQ ID NO:8) containing an Xho I restriction site. The mixture was heated at 70°C for 4 minutes and cooled by chilling on ice. First stand cDNA synthesis was initiated by the addition of 12 µl of first strand buffer (5x SUPERScript™  
30 buffer; Life Technologies, Gaithersburgh, MD), 6 µl of 100 mM dithiothreitol, 3 µl of deoxynucleotide triphosphate solution containing 10 mM each of dTTP, dATP, dGTP, and 5-methyl-dCTP (Pharmacia LKB Biotechnology, Piscataway, NJ) to the RNA-primer mixture. The reaction mixture was  
35 incubated at 37°C for 2 minutes, followed by the addition of 15 µl of 200 U/µl Rnase H<sup>-</sup> reverse transcriptase

(SUPERScript II<sup>®</sup> ; Life Technologies). The efficiency of the first strand synthesis was analyzed in a parallel reaction by the addition of 5  $\mu$ Ci of <sup>32</sup>P- $\alpha$ dCTP to 5  $\mu$ l aliquot from one of the reaction mixtures to label the  
5 reaction for analysis. The reactions were incubated at 37°C for 10 minutes, 45°C for 1 hour, then incubated at 50°C for 10 minutes. Unincorporated <sup>32</sup>P- $\alpha$ dCTP in the labeled reaction and the unincorporated nucleotides and primers in the unlabeled first strand reactions were removed by  
10 chromatography on a 400 pore size gel filtration column (Clontech Laboratories). The length of labeled first strand cDNA was determined by agarose gel electrophoresis.

The second strand reaction contained 120  $\mu$ l of the  
15 unlabeled first strand cDNA, 36  $\mu$ l of 5x polymerase I buffer (125 mM Tris: HCl, pH 7.5, 500 mM KCl, 25 mM MgCl<sub>2</sub>, 50mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), 2.4  $\mu$ l of 100 mM dithiothreitol, 3.6  $\mu$ l of a solution containing 10 mM of each deoxynucleotide triphosphate, 6  $\mu$ l of 5 mM  $\beta$ -NAD, 3.6  $\mu$ l of 3 U/ $\mu$ l *E. coli*  
20 DNA ligase (New England Biolabs), 9  $\mu$ l of 10 U/ $\mu$ l *E. coli* DNA polymerase I (New England Biolabs), and 1.8  $\mu$ l of 2 U/ $\mu$ l RNase H (Life Technologies). A 10  $\mu$ l aliquot from one of the second strand synthesis reactions was labeled by the addition of 10  $\mu$ Ci <sup>32</sup>P- $\alpha$ dCTP to monitor the efficiency  
25 of second strand synthesis. The reactions were incubated at 16°C for two hours, followed by the addition of 15  $\mu$ l T4 DNA polymerase (10 U/ $\mu$ l, Boehringer Mannheim, Indianapolis, IN) and incubated for an additional 5 minutes at 16°C. Unincorporated <sup>32</sup>P- $\alpha$ dCTP in the labeled  
30 reaction was removed by chromatography through a 400 pore size gel filtration (Clontech Laboratories) before analysis by agarose gel electrophoresis. The unlabeled second strand reaction was terminated by the addition of 20  $\mu$ l 0.5 M EDTA and extraction with phenol/chloroform and  
35 chloroform followed by ethanol precipitation in the presence of 2.5 M ammonium acetate and 4  $\mu$ g of glycogen



carrier. The yield of cDNA was estimated to be approximately 3 µg from starting mRNA template of 15 µg.

Eco RI adapters were ligated onto the 5'ends of the  
5 cDNA described above to enable cloning into an expression  
vector. A 10 µl aliquot of cDNA (approximately 1.5 µg) and  
5 µl of 65 pmole/µl of Eco RI adapter (Pharmacia LKB  
Biotechnology Inc.) were mixed with 2 µl 10x ligase buffer  
(660 mM Tris-HCl pH 7.5, 100 mM MgCl<sub>2</sub>), 2 µl of 10 mM ATP  
10 and 1 µl of 15 U/µl T4 DNA ligase (Promega Corp., Madison,  
WI). The reaction was incubated 2 hours at 5°C, two hours  
at 7.5°C, 2 hours at 10°C, and 10 hours at 12.5°C. The  
reaction was terminated by incubation at 70°C for 20  
minutes.

15

To facilitate the directional cloning of the cDNA  
into an expression vector, the cDNA was digested with *Xho*  
I, resulting in a cDNA having a 5' Eco RI cohesive end and  
a 3' *Xho* cohesive end. The *Xho* I restriction site at the  
20 3'end of the cDNA had been previously introduced using the  
ZC6091 primer (SEQ ID NO: 8). Restriction enzyme digestion  
was carried out in a reaction mixture containing 20 µl of  
cDNA as described above, 10 µl of 10x H Buffer *Xho* I  
(Boehringer Mannheim), 69 µl H<sub>2</sub>O, and 1.0 µl of 40 U/µl *Xho*  
25 I (Boehringer Mannheim). Digestion was carried out at 37°C  
for 40 minutes. The reaction was terminated by incubation  
at 70°C for 10 minutes and chromatography through a 400  
pore size gel filtration column (Clontech Laboratories).

30 The cDNA was ethanol precipitated, washed with 70%  
ethanol, air dried and resuspended in 14 µl water, 2 µl of  
ligase buffer (Promega Corp., Madison, WI), 2 µl T4  
polynucleotide kinase (10 U/µl, Life Technologies).  
Following incubation at 37°C for 30 minutes, the cDNA was  
35 heated to 65°C for 5 minutes, cooled on ice, and  
electrophoresed on a 0.8% low melt agarose gel. The

contaminating adapters and cDNA below 0.6 kb in length were excised from the gel. The electrodes were reversed, and the cDNA was electrophoresed until concentrated near the lane origin. The area of the gel containing the concentrated cDNA was excised and placed in a microfuge tube, and the approximate volume of the gel slice was determined. An aliquot of water approximately three times the volume of the gel slice (300  $\mu$ l) and 35  $\mu$ l 10x  $\beta$ -agarose I buffer (New England Biolabs) were added to the tube, and the agarose was melted by heating to 65°C for 15 minutes. Following equilibration of the sample to 45°C, 3  $\mu$ l of 1 U/ $\mu$ l  $\beta$ -agarose I (New England Biolabs) was added, and the mixture was incubated for 60 minutes at 45°C to digest the agarose. After incubation, 40  $\mu$ l of 3 M Na acetate was added to the sample, and the mixture was incubated on ice for 15 minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose. The cDNA was ethanol precipitated, washed in 70% ethanol, air-dried and resuspended in 10  $\mu$ l water.

The resulting cDNA was cloned into the lambda phage vector  $\lambda$ Zap<sup>®</sup> II (Stratagene Cloning Systems) that was predigested with *Eco* RI and *Xho* I and dephosphorylated. Ligation of the cDNA to the  $\lambda$ Zap<sup>®</sup> II vector was carried out in a reaction mixture containing 1.0  $\mu$ l of prepared vector, 1.0  $\mu$ l of human testis cDNA, 1.0  $\mu$ l 10X Ligase Buffer (Promega Corp.), 1.0  $\mu$ l of 10 mM ATP, 5  $\mu$ l water, and 1.0  $\mu$ l of T4 DNA Ligase at 15 units/ml (Promega Corp.). The ligation mixture was incubated at 5°-15°C overnight in a temperature gradient. After incubation, the ligation mixture was packaged into phage using an *in vitro* packaging extract (Gigapack<sup>®</sup> III Gold packaging extract; Stratagene Cloning Systems), and the resulting library was titered according to the manufacturer's specifications.

The human testis  $\lambda$ ZAP<sup>®</sup> II library was used to infect *E. coli* host cells (XL1-Blue MRF' strain (Stratagene Cloning Systems), and  $1.5 \times 10^6$  plaque forming units (pfu) were plated onto 150-mm NZY plates at a density of about 50,000 pfu/plate. The inoculated plates were incubated overnight at 37°C. Filter plaque lifts were made using nylon membranes (Hybond<sup>™</sup>-N; Amersham Corp., Arlington Heights, IL), according to the procedures provided by the manufacturer. The filters were processed by denaturation in solution containing 1.5 M NaCl and 0.5 M NaOH for 6 minutes at room temperature. The filters were blotted briefly on filter paper to remove excess denaturation solution, followed by neutralization for 6 minutes in 1 M Tris-HCl, pH 7.5, and 1.5 M NaCl. Phage DNA was fixed onto the filters with 1,200  $\mu$ Joules of UV energy in a UV Crosslinker (Stratalinker<sup>®</sup>; Stratagene Cloning Systems). After fixing, the filters were first pre-washed in an aqueous solution containing .25X standard sodium citrate (SSC), .25% sodium dodecyl sulfate (SDS) and 1mM EDTA to remove cellular debris and then prehybridized in hybridization solution (5X SSC, 5X Denhardt's solution, 0.2% SDS and 1 mM EDTA). Heat-denatured, sheared salmon sperm DNA at a final concentration of 100  $\mu$ g/ml was added. The filters were prehybridized at 65°C overnight.

25

A probe was prepared as a PCR product by using oligonucleotide primers designed to amplify the human Zcytor7 coding region. A PCR reaction mixture was prepared containing 2  $\mu$ l of ZC11526 (SEQ ID NO:9) 2  $\mu$ l of ZC11,108 (SEQ ID NO:6), 1  $\mu$ l of an overnight bacterial culture of pSLR7-1, 1  $\mu$ l of 10 mM dNTP, 10  $\mu$ l of 10X KlenTaq buffer (Clontech Laboratories), 82  $\mu$ l water, and 2  $\mu$ l KlenTaq DNA polymerase (Clontech laboratories). The PCR reaction was run as follows: 94°C for 1 minute; 30 cycles of 95°C for 20 seconds, 43°C for 20 seconds, 68°C for 1 minute; then held

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at 68°C for 10 minutes. The PCR product was re-amplified and gel purified on a .8% low melt agarose gel.

Fifty nanograms PCR product was radiolabeled with  
5  $^{32}\text{P}$ - $\alpha$ -dCTP by random priming using the MEGAPRIME® DNA  
Labeling System (Amersham), according to the  
manufacturer's specifications. The prehybridization  
solution was replaced with fresh hybridization solution  
containing  $1.4 \times 10^6$  cpm/ml labeled probe and allowed to  
10 hybridize for 64 hours at 60°C. After hybridization, the  
hybridization solution was removed and the filters were  
rinsed in a wash solution containing .25X SSC, 0.25% SDS  
and 1 mM EDTA at 65°C. The filters were placed on  
autoradiograph film and exposed at -70°C with intensifying  
15 screens for 72 hours.

Examination of the autoradiographs revealed multiple  
regions that hybridized with labeled probe. Agar plugs  
were picked from 12 regions for purification. Each agar  
20 plug was soaked 2 hours in 0.5 ml of SM solution  
containing 25 ml 4M NaCl, 10 ml 1M  $\text{MgSO}_4$ , 25 ml 2M Tris  
HCl, 5 ml 2% gelatin and 935 ml  $\text{H}_2\text{O}$  and 10% (v/v)  
chloroform (Sambrook et al. *Molecular Cloning: A  
Laboratory Manual*, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory  
25 Press, Cold Spring Harbor, NY, 1989). After incubation,  
the phages from each plug were diluted 1:1000 in SM.  
Aliquots of 50  $\mu\text{l}$  were plated on 100 mm plates containing  
300  $\mu\text{l}$  of *E. coli* XL-1 Blue MRF' cells. The plates were  
incubated overnight at 37°C, and filter lifts were  
30 prepared, prehybridized overnight, hybridized overnight  
with a hybridization solution containing  $1.1 \times 10^6$  cpm/ml  
labeled probe, washed and autoradiographed. Examination of  
the resulting autoradiographs revealed 10 positive  
signals. The positive plaques were subjected to an  
35 additional round of purification.

The plasmids were excised using an ExASSIST/SOLR<sup>®</sup> system (Stratagene Cloning Systems), according to the manufacturer's specifications. These plasmid inserts were amplified by PCR for size determination. A clone,  
5 designated pSLR7-2 was sequenced and determined to have an insert of 3,532 bp in size.

### Example 2

#### Northern Blot Analysis

10

A 970 bp fragment of the Zcytor7 cDNA containing nucleotides 822 - 1791 was random primer labeled using a MULTIPRIME<sup>™</sup> kit (Amersham Corp.). Labeled cDNA was purified from free counts using a push column (Stratagene  
15 Cloning Systems). A human RNA master dot blot (Clontech Laboratories) for three hours at 65°C, then hybridized with 10<sup>6</sup>cpm/ml of labeled cDNA probe. The expression pattern for this blot, which contained RNA samples which had been normalized to the mRNA expression levels of eight  
20 different housekeeping genes, was highest in kidney, followed by spinal cord, prostate, and cerebellum.

### Example 3

#### Expression of Human Zcytor7 mRNA in Human Tissues

25

Poly(A)<sup>+</sup> RNAs isolated from adrenal cortex, adrenal medulla, brain, colon, heart, kidney, liver, lung, ovary, pancreas, prostate, placenta, peripheral blood leukocytes, stomach, spleen, skeletal muscle, small intestine, testis,  
30 thymus, thyroid, fetal brain, fetal lung, fetal liver and fetal kidney were hybridized under high stringency conditions with a radiolabeled DNA probe containing nucleotides 822-1791 of (SEQ ID NO:1). Membranes were purchased from Clontech. The membrane were washed with  
35 0.1X SSC, 0.1% SDS at 50°C and autoradiographed for 24 hours. The mRNA levels were highest in adrenal cortex,

pancreas and prostate with lower levels in testis, stomach, adrenal medulla and thyroid.

#### Example 4

5

#### Chromosomal Assignment and Placement of Zcytor-7

Zcytor-7 was mapped to chromosome 6 using the commercially available version of the Whitehead  
10 Institute/MIT Center for Genome Research's "GeneBridge 4 Radiation Hybrid Panel" (Research Genetics, Inc., Huntsville, AL). The GeneBridge 4 Radiation Hybrid Panel contains PCRable DNAs from each of 93 radiation hybrid clones, plus two control DNAs (the HFL donor and the A23  
15 recipient). A publicly available WWW server (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>) allows mapping relative to the Whitehead Institute/MIT Center for Genome Research's radiation hybrid map of the human genome (the "WICGR" radiation hybrid map) which was constructed  
20 with the GeneBridge 4 Radiation Hybrid Panel.

For the mapping of zcytor-7 with the "GeneBridge 4 RH Panel", 25 µl reactions were set up in a PCRable 96-well microtiter plate (Stratagene, La Jolla, CA) and used in a  
25 "RoboCycler Gradient 96" thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2.5 µl 50X "Advantage KlenTaq Polymerase Mix" (CLONTECH Laboratories, Inc., Palo Alto, CA), 2 µl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1.25 µl sense primer, SEQ ID NO: 11,  
30 1.25 µl antisense primer SEQ ID NO: 12, 2.5 µl "RediLoad" (Research Genetics, Inc., Huntsville, AL), 0.5 µl "Advantage KlenTaq Polymerase Mix" (Clontech Laboratories, Inc.), 25 ng of DNA from an individual hybrid clone or control and water is added to bring up the total volume to  
35 25 µl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as

follows: an initial 1 cycle 4 minute denaturation at 94°C, 35 cycles of a 1 minute denaturation at 94°C, 1.5 minute annealing at 63°C and 1.5 minute extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C.

5 The reactions were separated by electrophoresis on a 3% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME).

The results showed that zcytor-7 maps 795.76 cR from the top of the human chromosome 6 linkage group on the

10 WICGR radiation hybrid map. Relative to the centromere, its nearest proximal marker was CHLC.GATA32B03 and its nearest distal marker was SGC32063. The use of surrounding markers also helped position zcytor-7 in the 6q22-q23 region on the CHLC chromosome 6 version v8c7 integrated

15 marker map (The Cooperative Human Linkage Center, WWW server: [http:// www.chlc.org/ChlcIntegratedMaps.html](http://www.chlc.org/ChlcIntegratedMaps.html)) and to 6q22.33-q23.1 on the integrated LDB chromosome 6 map (The Genetic Location Database, University of Southhampton, WWW

20 server:[http://cedar.genetics.soton.ac.uk/public\\_html/](http://cedar.genetics.soton.ac.uk/public_html/)).

This is a common breakpoint area in ALL(acute lymphoblastic leukemia) and NHL(non-Hodgkin lymphoma) as well as in AML(acute myelogenous leukemia) and CML(chronic

25 myeloid leukemia). It is interesting to note that the MYB (avian myeloblastosis viral oncogene homolog) gene, which encodes proteins critical for hematopoietic cell proliferation and development, appears to be less than 800 kB from Zcytor7. The 6q- deletion breakpoints occur

30 slightly distal to the MYB gene and although the neoplasms show high levels of MYB mRNA, the gene itself appears to be intact.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: ZymoGenetic, Inc.  
1201 Eastlake Avenue East  
Seattle  
WA  
USA  
98102
- (ii) TITLE OF THE INVENTION: CYTOKINE RECEPTOR
- (iii) NUMBER OF SEQUENCES: 13
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: ZymoGenetics, Inc.
  - (B) STREET: 1201 Eastlake Avenue East
  - (C) CITY: Seattle
  - (D) STATE: WA
  - (E) COUNTRY: USA
  - (F) ZIP: 98102
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: DOS
  - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/803,305
  - (B) FILING DATE: 20-FEB-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Lunn, Paul G
  - (B) REGISTRATION NUMBER: 32,743



(C) REFERENCE/DOCKET NUMBER: 96-24PC

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 206-442-6627
- (B) TELEFAX: 206-442-6678
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3516 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 237...1895
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCCAGCTGGG TAGCCGGGGG AGCGCGCGTG GGGGCTCCGC GAGTCGCTCG CCCTTGTTTT	60
CTGGGGAAGC CTGGGGGACG CGGCTGTGGC GGAGGCGCCC TGGGACTCAG GTCGCCTGGA	120
GCGTGGCAGC CAGAGCCCCA GGCGCGGAGC TGAGGCCGCG CGGCCGCGCT TGGCCCCAGC	180
GGGCGTGGGA CTGAGCAGTC TGCTGCCCCC CGACATGTGA CCCAGCCCCG CCGCCC ATG	239
	Met
	1
CGG GCT CCC GGC CGC CCG GCC CTG CGG CCG CTG CCG CTG CCG CCG CTG	287
Arg Ala Pro Gly Arg Pro Ala Leu Arg Pro Leu Pro Leu Pro Pro Leu	
5 10 15	
CTG CTG TTG CTC CTG GCG GCG CCT TGG GGA CGG GCA GTT CCC TGT GTC	335
Leu Leu Leu Leu Leu Ala Ala Pro Trp Gly Arg Ala Val Pro Cys Val	
20 25 30	
TCT GGT GGT TTG CCT AAA CCT GCA AAC ATC ACC TTC TTA TCC ATC AAC	383
Ser Gly Gly Leu Pro Lys Pro Ala Asn Ile Thr Phe Leu Ser Ile Asn	
35 40 45	

ATG AAG AAT GTC CTA CAA TGG ACT CCA CCA GAG GGT CTT CAA GGA GTT Met Lys Asn Val Leu Gln Trp Thr Pro Pro Glu Gly Leu Gln Gly Val 50 55 60 65	431
AAA GTT ACT TAC ACT GTG CAG TAT TTC ATA TAT GGG CAA AAG AAA TGG Lys Val Thr Tyr Thr Val Gln Tyr Phe Ile Tyr Gly Gln Lys Lys Trp 70 75 80	479
CTG AAT AAA TCA GAA TGC AGA AAT ATC AAT AGA ACC TAC TGT GAT CTT Leu Asn Lys Ser Glu Cys Arg Asn Ile Asn Arg Thr Tyr Cys Asp Leu 85 90 95	527
TCT GCT GAA ACT TCT GAC TAC GAA CAC CAG TAT TAT GCC AAA GTT AAG Ser Ala Glu Thr Ser Asp Tyr Glu His Gln Tyr Tyr Ala Lys Val Lys 100 105 110	575
GCC ATT TGG GGA ACA AAG TGT TCC AAA TGG GCT GAA AGT GGA CGG TTC Ala Ile Trp Gly Thr Lys Cys Ser Lys Trp Ala Glu Ser Gly Arg Phe 115 120 125	623
TAT CCT TTT TTA GAA ACA CAA ATT GGC CCA CCA GAG GTG GCA CTG ACT Tyr Pro Phe Leu Glu Thr Gln Ile Gly Pro Pro Glu Val Ala Leu Thr 130 135 140 145	671
ACA GAT GAG AAG TCC ATT TCT GTT GTC CTG ACA GCT CCA GAG AAG TGG Thr Asp Glu Lys Ser Ile Ser Val Val Leu Thr Ala Pro Glu Lys Trp 150 155 160	719
AAG AGA AAT CCA GAA GAC CTT CCT GTT TCC ATG CAA CAA ATA TAC TCC Lys Arg Asn Pro Glu Asp Leu Pro Val Ser Met Gln Gln Ile Tyr Ser 165 170 175	767
AAT CTG AAG TAT AAC GTG TCT GTG TTG AAT ACT AAA TCA AAC AGA ACG Asn Leu Lys Tyr Asn Val Ser Val Leu Asn Thr Lys Ser Asn Arg Thr 180 185 190	815
TGG TCC CAG TGT GTG ACC AAC CAC ACG CTG GTG CTC ACC TGG CTG GAG Trp Ser Gln Cys Val Thr Asn His Thr Leu Val Leu Thr Trp Leu Glu 195 200 205	863
CCG AAC ACT CTT TAC TGC GTA CAC GTG GAG TCC TTC GTC CCA GGG CCC Pro Asn Thr Leu Tyr Cys Val His Val Glu Ser Phe Val Pro Gly Pro 210 215 220 225	911

CCT CGC CGT GCT CAG CCT TCT GAG AAG CAG TGT GCC AGG ACT TTG AAA	959
Pro Arg Arg Ala Gln Pro Ser Glu Lys Gln Cys Ala Arg Thr Leu Lys	
230 235 240	
GAT CAA TCA TCA GAG TTC AAG GCT AAA ATC ATC TTC TGG TAT GTT TTG	1007
Asp Gln Ser Ser Glu Phe Lys Ala Lys Ile Ile Phe Trp Tyr Val Leu	
245 250 255	
CCC ATA TCT ATT ACC GTG TTT CTT TTT TCT GTG ATG GGC TAT TCC ATC	1055
Pro Ile Ser Ile Thr Val Phe Leu Phe Ser Val Met Gly Tyr Ser Ile	
260 265 270	
TAC CGA TAT ATC CAC GTT GGC AAA GAG AAA CAC CCA GCA AAT TTG ATT	1103
Tyr Arg Tyr Ile His Val Gly Lys Glu Lys His Pro Ala Asn Leu Ile	
275 280 285	
TTG ATT TAT GGA AAT GAA TTT GAC AAA AGA TTC TTT GTG CCT GCT GAA	1151
Leu Ile Tyr Gly Asn Glu Phe Asp Lys Arg Phe Phe Val Pro Ala Glu	
290 295 300 305	
AAA ATC GTG ATT AAC TTT ATC ACC CTC AAT ATC TCG GAT GAT TCT AAA	1199
Lys Ile Val Ile Asn Phe Ile Thr Leu Asn Ile Ser Asp Asp Ser Lys	
310 315 320	
ATT TCT CAT CAG GAT ATG AGT TTA CTG GGA AAA AGC AGT GAT GTA TCC	1247
Ile Ser His Gln Asp Met Ser Leu Leu Gly Lys Ser Ser Asp Val Ser	
325 330 335	
AGC CTT AAT GAT CCT CAG CCC AGC GGG AAC CTG AGG CCC CCT CAG GAG	1295
Ser Leu Asn Asp Pro Gln Pro Ser Gly Asn Leu Arg Pro Pro Gln Glu	
340 345 350	
GAA GAG GAG GTG AAA CAT TTA GGG TAT GCT TCG CAT TTG ATG GAA ATT	1343
Glu Glu Glu Val Lys His Leu Gly Tyr Ala Ser His Leu Met Glu Ile	
355 360 365	
TTT TGT GAC TCT GAA GAA AAC ACG GAA GGT ACT TCT TTC ACC CAG CAA	1391
Phe Cys Asp Ser Glu Glu Asn Thr Glu Gly Thr Ser Phe Thr Gln Gln	
370 375 380 385	
GAG TCC CTC AGC AGA ACA ATA CCC CCG GAT AAA ACA GTC ATT GAA TAT	1439
Glu Ser Leu Ser Arg Thr Ile Pro Pro Asp Lys Thr Val Ile Glu Tyr	
390 395 400	

GAA TAT GAT GTC AGA ACC ACT GAC ATT TGT GCG GGG CCT GAA GAG CAG Glu Tyr Asp Val Arg Thr Thr Asp Ile Cys Ala Gly Pro Glu Glu Gln 405 410 415	1487
GAG CTC AGT TTG CAG GAG GAG GTG TCC ACA CAA GGA ACA TTA TTG GAG Glu Leu Ser Leu Gln Glu Glu Val Ser Thr Gln Gly Thr Leu Leu Glu 420 425 430	1535
TCG CAG GCA GCG TTG GCA GTC TTG GGC CCG CAA ACG TTA CAG TAC TCA Ser Gln Ala Ala Leu Ala Val Leu Gly Pro Gln Thr Leu Gln Tyr Ser 435 440 445	1583
TAC ACC CCT CAG CTC CAA GAC TTA GAC CCC CTG GCG CAG GAG CAC ACA Tyr Thr Pro Gln Leu Gln Asp Leu Asp Pro Leu Ala Gln Glu His Thr 450 455 460 465	1631
GAC TCG GAG GAG GGG CCG GAG GAA GAG CCA TCG ACG ACC CTG GTC GAC Asp Ser Glu Glu Gly Pro Glu Glu Glu Pro Ser Thr Thr Leu Val Asp 470 475 480	1679
TGG GAT CCC CAA ACT GGC AGG CTG TGT ATT CCT TCG CTG TCC AGC TTC Trp Asp Pro Gln Thr Gly Arg Leu Cys Ile Pro Ser Leu Ser Ser Phe 485 490 495	1727
GAC CAG GAT TCA GAG GGC TGC GAG CCT TCT GAG GGG GAT GGG CTC GGA Asp Gln Asp Ser Glu Gly Cys Glu Pro Ser Glu Gly Asp Gly Leu Gly 500 505 510	1775
GAG GAG GGT CTT CTA TCT AGA CTC TAT GAG GAG CCG GCT CCA GAC AGG Glu Glu Gly Leu Leu Ser Arg Leu Tyr Glu Glu Pro Ala Pro Asp Arg 515 520 525	1823
CCA CCA GGA GAA AAT GAA ACC TAT CTC ATG CAA TTC ATG GAG GAA TGG Pro Pro Gly Glu Asn Glu Thr Tyr Leu Met Gln Phe Met Glu Glu Trp 530 535 540 545	1871
GGG TTA TAT GTG CAG ATG GAA AAC TGATGCCAAC ACTTCCTTTT GCCTTTTGT Gly Leu Tyr Val Gln Met Glu Asn 550	1925
TCCTGTGCAA ACAAGTGAGT CACCCCTTTG ATCCCAGCCA TAAAGTACCT GGGATGAAAG AAGTTTTTTC CAGTTTGTCA GTGTCTGTGA GAATTACTTA TTTCTTTTCT CTATTCTCAT AGCACGTGTG TGATTGGTTC ATGCATGTAG GTCTCTTAAC AATGATGGTG GGCCTCTGGA GTCCAGGGGC TGGCCGGTTG TTCTATGCAG AGAAAGCAGT CAATAAATGT TTGCCAGACT GGGTGCAGAA TTTATTCAGG TGGGTGTACT CTGGCCTCTT GGTTCATTAT TTCAAACAA 2225	1985 2045 2105 2165 2225

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GCACACTTGT ACAATTATTT TCTGGGTACT TCCCATATGC ACATAGCACT GTAAAAAATA 2285
TTTCCCAAAG ATCACTCATT TTATAAATAC CACTTTTTCA GAATTGGGTT TATTGCGAGC 2345
AGGAGGAGAT ACTTAAACA TGCACATATA CCAGGTTGGT GGTAAGTTGG TCACATGTGA 2405
AAACCTCAAC TATTTAATCA TCATGATTCA TATTTTGAGT GAATACATCA GGCACAGACC 2465
TTCATGATAT CACACACTCT TGGCTACTTT AAGAGGCCAT CTTTAATACT TTATGAGTAG 2525
TTCTGGAGTG TAAACATAAA CGAGTATTCT TTTGTAGTCA GAAAAGTGTC CTCTCAATAA 2585
TTTAGTAGGG GCTTATTGTC TCTCAAACCT AACCTAAAAG AAAATGACAC ATTTTATAAT 2645
AGAATATTAC ATTTATTTCT GGAAGTGTGT TTTCAAAAAG ATATTTACAT AGTCTGTAAA 2705
CTAGAAAGTG TTAGGTAAAG CTCTAGGTTA CTGTGTTACT ATTATAATAT TAAACATTCTG 2765
AATAGGCAGT CGTTCAAAGA CTCTTTGGAA TATCTATGAA TGAATATCCT CTATTCTTAT 2825
AATATTAATA CCCATAAGTA AATATAGGAC ATACAAGAGA AATGAGTTAA ATGACTATGT 2885
AAGGGAGAGT TTATTAAAT TTGATGAAAT TTAGTGTAGG AACTAAACTA TGCCATAAAA 2945
CAATAGCTTT CTAGTTCATT TCCAGTAACT GTTCCCCTCT CCTTTACCAC TTGTAAAGAA 3005
AATTAAATTC TTCAGTCACG CTGCTTTAAA ATGGGACAAA ATCTATTAAG TTGAACCATA 3065
TATAATTGTG GATATTTGGC TGTTTTTAAT CTGACAAGCA GTAAC TTCAT ATGGTTTGCC 3125
TTAATATATA TTTGTTTTAG TCATGAAGTC ATAATCCATT GATGCTCTTT CATGAGAAGA 3185
GATATGACCC ATATTTCTTT ATTGATATTA TTGGTACAGG CAGACAACCC TGGTAGGAGA 3245
GATGGATTCT GGGGTCATGA CCTTCGTGA TTATCCGCAA ATGCAAACAG TTTCAGATCT 3305
AATGGTTTAA TTTAGGGAGT AATTATATTA ATCAGAGTGT TCTGTTATTC TCAATCTTTA 3365
TAGAAACGAT TCTGCTGGTT TTGAAGAACA GATGTATTAC ACTAACTGTA AAAGTAGTTC 3425
AAGAGTGAGA AAGAATAAAT TGTTATTAAG AGCAAAAAGAA AAATAAAGTG ATTGATGATA 3485
AAAAAAAAAA AAAAAAAGCG GCCGCCTCGA G 3516

```

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 553 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Arg Ala Pro Gly Arg Pro Ala Leu Arg Pro Leu Pro Leu Pro Pro
 1           5           10           15
Leu Leu Leu Leu Leu Ala Ala Pro Trp Gly Arg Ala Val Pro Cys
          20          25          30
Val Ser Gly Gly Leu Pro Lys Pro Ala Asn Ile Thr Phe Leu Ser Ile
          35          40          45
Asn Met Lys Asn Val Leu Gln Trp Thr Pro Pro Glu Gly Leu Gln Gly
          50          55          60

```

60

Val	Lys	Val	Thr	Tyr	Thr	Val	Gln	Tyr	Phe	Ile	Tyr	Gly	Gln	Lys	Lys
65					70					75					80
Trp	Leu	Asn	Lys	Ser	Glu	Cys	Arg	Asn	Ile	Asn	Arg	Thr	Tyr	Cys	Asp
				85					90					95	
Leu	Ser	Ala	Glu	Thr	Ser	Asp	Tyr	Glu	His	Gln	Tyr	Tyr	Ala	Lys	Val
			100					105					110		
Lys	Ala	Ile	Trp	Gly	Thr	Lys	Cys	Ser	Lys	Trp	Ala	Glu	Ser	Gly	Arg
			115				120					125			
Phe	Tyr	Pro	Phe	Leu	Glu	Thr	Gln	Ile	Gly	Pro	Pro	Glu	Val	Ala	Leu
					130		135				140				
Thr	Thr	Asp	Glu	Lys	Ser	Ile	Ser	Val	Val	Leu	Thr	Ala	Pro	Glu	Lys
145					150					155					160
Trp	Lys	Arg	Asn	Pro	Glu	Asp	Leu	Pro	Val	Ser	Met	Gln	Gln	Ile	Tyr
				165						170				175	
Ser	Asn	Leu	Lys	Tyr	Asn	Val	Ser	Val	Leu	Asn	Thr	Lys	Ser	Asn	Arg
			180					185					190		
Thr	Trp	Ser	Gln	Cys	Val	Thr	Asn	His	Thr	Leu	Val	Leu	Thr	Trp	Leu
			195				200					205			
Glu	Pro	Asn	Thr	Leu	Tyr	Cys	Val	His	Val	Glu	Ser	Phe	Val	Pro	Gly
			210			215					220				
Pro	Pro	Arg	Arg	Ala	Gln	Pro	Ser	Glu	Lys	Gln	Cys	Ala	Arg	Thr	Leu
225					230					235					240
Lys	Asp	Gln	Ser	Ser	Glu	Phe	Lys	Ala	Lys	Ile	Ile	Phe	Trp	Tyr	Val
				245					250					255	
Leu	Pro	Ile	Ser	Ile	Thr	Val	Phe	Leu	Phe	Ser	Val	Met	Gly	Tyr	Ser
			260				265						270		
Ile	Tyr	Arg	Tyr	Ile	His	Val	Gly	Lys	Glu	Lys	His	Pro	Ala	Asn	Leu
		275				280						285			
Ile	Leu	Ile	Tyr	Gly	Asn	Glu	Phe	Asp	Lys	Arg	Phe	Phe	Val	Pro	Ala
		290				295					300				
Glu	Lys	Ile	Val	Ile	Asn	Phe	Ile	Thr	Leu	Asn	Ile	Ser	Asp	Asp	Ser
305					310					315					320
Lys	Ile	Ser	His	Gln	Asp	Met	Ser	Leu	Leu	Gly	Lys	Ser	Ser	Asp	Val
				325					330					335	
Ser	Ser	Leu	Asn	Asp	Pro	Gln	Pro	Ser	Gly	Asn	Leu	Arg	Pro	Pro	Gln
			340					345					350		
Glu	Glu	Glu	Glu	Val	Lys	His	Leu	Gly	Tyr	Ala	Ser	His	Leu	Met	Glu
			355				360					365			
Ile	Phe	Cys	Asp	Ser	Glu	Glu	Asn	Thr	Glu	Gly	Thr	Ser	Phe	Thr	Gln
			370				375				380				
Gln	Glu	Ser	Leu	Ser	Arg	Thr	Ile	Pro	Pro	Asp	Lys	Thr	Val	Ile	Glu
385					390					395					400
Tyr	Glu	Tyr	Asp	Val	Arg	Thr	Thr	Asp	Ile	Cys	Ala	Gly	Pro	Glu	Glu
				405					410					415	

61

Gln Glu Leu Ser Leu Gln Glu Glu Val Ser Thr Gln Gly Thr Leu Leu  
 420 425 430  
 Glu Ser Gln Ala Ala Leu Ala Val Leu Gly Pro Gln Thr Leu Gln Tyr  
 435 440 445  
 Ser Tyr Thr Pro Gln Leu Gln Asp Leu Asp Pro Leu Ala Gln Glu His  
 450 455 460  
 Thr Asp Ser Glu Glu Gly Pro Glu Glu Glu Pro Ser Thr Thr Leu Val  
 465 470 475 480  
 Asp Trp Asp Pro Gln Thr Gly Arg Leu Cys Ile Pro Ser Leu Ser Ser  
 485 490 495  
 Phe Asp Gln Asp Ser Glu Gly Cys Glu Pro Ser Glu Gly Asp Gly Leu  
 500 505 510  
 Gly Glu Glu Gly Leu Leu Ser Arg Leu Tyr Glu Glu Pro Ala Pro Asp  
 515 520 525  
 Arg Pro Pro Gly Glu Asn Glu Thr Tyr Leu Met Gln Phe Met Glu Glu  
 530 535 540  
 Trp Gly Leu Tyr Val Gln Met Glu Asn  
 545 550

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 451 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Other

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGTGTGTGAC CAACCACACG CTGGTGCTCA CCTGGGCTGG AGCCGAACAC TCTTTACTGC	60
GTACACGTGG AGTCCTTCGT CCCAGGGCCC CCTCGCCGTG CTCAGCCTTC TGAGAAGCAG	120
TGTGCCAGGA CTTTGAAAGA TCAATCATCA GAGTTCAAGG CTAAAATCAT CTTCTGGTAT	180
GTTTTGCCCA TATCTATTAC CGTGTTTCTT TTTTCTGTGA TGGGCTATTC CATCTACCGA	240
TATATCCACG TTGGGCAAAG AGAAACACCC AGGCAAATTT GATTTTGATT TATGGGAAAT	300
GAATTTGACA AAAGATTCTT TGTGCCTGCT GGAAAAATC GTGGATTAAC TTTATTCACC	360
CTCAATATCT CGGGTGGATT CTAAAATTTT CTCCATCCAG GGGTATGGAG GTTTACTGGG	420
GGTAAANGCG GGTGTTGTTT NCCAGGCCTT A	451

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid

62

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: ZC11107

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCTGGGTGTT TCTCTTTG

18

- (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCCTAAT ACGACTCACT ATAGGGC

27

- (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: ZC11108

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAATAGCCC ATCACAGAAA

20

- (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs



63

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACTCACTATA GGGCTCGAGC GGC

23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC6091

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGCACAGAA TTCACTACTC GAGGCGGCCG CTTTTTTTTT TTTTTTTTTT

49

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC11526

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CACCAGAGGG TCTTCAAGGA GT

22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

64

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 3...3
- (D) OTHER INFORMATION: Xaa = any amino acid

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Trp Ser Xaa Trp Ser  
1 5

- (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGATCCTTTG TGCCTGCTGA

20

- (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTATTGTTCT GCTGAGGGAC

20

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 221 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Val	Pro	Cys	Val	Ser	Gly	Gly	Leu	Pro	Lys	Pro	Ala	Asn	Ile	Thr	Phe
1				5				10					15		
Leu	Ser	Ile	Asn	Met	Lys	Asn	Val	Leu	Gln	Trp	Thr	Pro	Pro	Glu	Gly
			20					25				30			
Leu	Gln	Gly	Val	Lys	Val	Thr	Tyr	Thr	Val	Gln	Tyr	Phe	Ile	Tyr	Gly
		35				40					45				
Gln	Lys	Lys	Trp	Leu	Asn	Lys	Ser	Glu	Cys	Arg	Asn	Ile	Asn	Arg	Thr
	50				55					60					
Tyr	Cys	Asp	Leu	Ser	Ala	Glu	Thr	Ser	Asp	Tyr	Glu	His	Gln	Tyr	Tyr
65					70				75					80	
Ala	Lys	Val	Lys	Ala	Ile	Trp	Gly	Thr	Lys	Cys	Ser	Lys	Trp	Ala	Glu
			85					90					95		
Ser	Gly	Arg	Phe	Tyr	Pro	Phe	Leu	Glu	Thr	Gln	Ile	Gly	Pro	Pro	Glu
			100					105					110		
Val	Ala	Leu	Thr	Thr	Asp	Glu	Lys	Ser	Ile	Ser	Val	Val	Leu	Thr	Ala
		115					120					125			
Pro	Glu	Lys	Trp	Lys	Arg	Asn	Pro	Glu	Asp	Leu	Pro	Val	Ser	Met	Gln
		130				135					140				
Gln	Ile	Tyr	Ser	Asn	Leu	Lys	Tyr	Asn	Val	Ser	Val	Leu	Asn	Thr	Lys
145				150					155					160	
Ser	Asn	Arg	Thr	Trp	Ser	Gln	Cys	Val	Thr	Asn	His	Thr	Leu	Val	Leu
			165					170					175		
Thr	Trp	Leu	Glu	Pro	Asn	Thr	Leu	Tyr	Cys	Val	His	Val	Glu	Ser	Phe
		180					185					190			
Val	Pro	Gly	Pro	Pro	Arg	Arg	Ala	Gln	Pro	Ser	Glu	Lys	Gln	Cys	Ala
		195					200					205			
Arg	Thr	Leu	Lys	Asp	Gln	Ser	Ser	Glu	Phe	Lys	Ala	Lys			
	210					215					220				

## CLAIMS

We claim:

1. An isolated polypeptide comprised of (a) a sequence of amino acid residues extending from residue 30, a valine, residue 250, a lysine, of SEQ ID NO: 2 (b) allelic variants of (a); and

(c) sequences that are at least 80% identical to (a) or (b),

2. The isolated polypeptide of claim 1 wherein the sequence of amino acid residues extends from residue 30, a valine, through residue 274, a tyrosine.

3. The isolated polypeptide of claim 1 wherein the sequence of amino acid residues extends from residue 30, a valine residue, through residue 553, an asparagine residue.

4. A polypeptide containing a subsequence of SEQ ID NO: 2 wherein said subsequence is at least 20 amino acids long.

5. A polypeptide of claim 1 further comprised of a transmembrane domain, an intracellular domain or both.

6. An isolated polynucleotide which encodes a polypeptide defined by claims 1-4.

7. An expression vector which contains the polynucleotide of claim 6 the following operably linked elements:

a transcription promoter;

a polynucleotide sequence of claim 5 and  
a transcription terminator.

8. A method for producing an antibody comprising:  
inoculating a polypeptide defined by SEQ ID NO: 2, a  
subsequence thereof or a polypeptide containing at least 20  
amino acids of SEQ ID NO: 2 to an animal wherein said animal  
produces antibodies which bind to the polypeptide of SEQ ID  
NO: 2; and

isolating said antibodies.

9. An antibody produced by the process of claim 7.

10. An anti-idiotypic antibody which binds to and  
neutralizes an antibody of claim 9.

11. An antibody which specifically binds to a  
polypeptide of claims 1-4.

12. A chimeric polypeptide consisting essentially  
of a first portion and a second portion joined by a peptide  
bond, said first portion consisting essentially of a ligand  
binding domain of a receptor polypeptide selected from the  
group consisting of:

(a) a receptor polypeptide as shown in SEQ ID NO:2  
containing residues 30-250;

(b) allelic variants of SEQ ID NO:2; and

(c) receptor polypeptides that are at least 80%  
identical to (a) or (b),

and said second portion consisting essentially of an affinity  
tag.

13. A polypeptide according to claim 12 wherein  
said affinity tag is an immunoglobulin F<sub>C</sub> polypeptide.

14. A method for detecting a ligand within a test  
sample, comprising contacting a test sample with a polypeptide  
comprising a segment selected from the group consisting of:

(a) residues 30 to 250 of SEQ ID NO:2;  
(b) allelic variants of (a); and  
(c) sequences that are at least 80% identical to  
(a) or (b),  
and detecting binding of said polypeptide to ligand  
in the sample.

15. A method according to claim 14 wherein said polypeptide further comprises transmembrane and intracellular domains.

16. A method according to claim 15 wherein said polypeptide is membrane bound within a cultured cell, and said detecting step comprises measuring a biological response in said cultured cell.

17. A method according to claim 16 wherein said biological response is cell proliferation or activation of transcription of a reporter gene.

18. A method according to claim 14 wherein said polypeptide is immobilized on a solid support.